

CLAIMS

1. A process for producing carboxylic acid, comprising converting at least one cyano group of a nitrile compound into a carboxyl group using a microorganism, wherein a variant microorganism defective or reduced in the activity of converting a cyano group into an amide group is used.
2. The process for producing carboxylic acid as claimed in claim 1, wherein said variant microorganism is a variant strain of a bacterium belonging to the genus *Rhodococcus*.
3. The process for producing carboxylic acid as claimed in claim 2, wherein said variant strain of a *Rhodococcus* bacterium is a variant strain of a parent strain *Rhodococcus* sp. ATCC39484.
4. The process for producing carboxylic acid as claimed in claim 3, wherein the variant strain of a parent strain *Rhodococcus* sp. ATCC39484 is *Rhodococcus* sp. SD826 (FERM BP-7305).
5. The process for producing carboxylic acid as claimed in claim 1, wherein the nitrile compound is a polynitrile compound having a plurality of cyano groups in the molecule and the carboxylic acid is a cyano carboxylic acid.
6. The process for producing carboxylic acid as claimed in claim 5, wherein the polynitrile compound is an aromatic polynitrile compound and the cyano carboxylic acid is an aromatic cyano carboxylic acid.
7. The process for producing carboxylic acid as claimed in claim 6, wherein the aromatic polynitrile compound is selected from the group consisting of o-phthalonitrile, isophthalonitrile, and terephthalonitrile, and the aromatic cyano carboxylic acid is

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corresponding o-cyanobenzoic acid, m-cyanobenzoic acid, or p-cyanobenzoic acid.

8. A variant microorganism having the activity of converting a cyano group into a carboxyl group and being defective or reduced in the activity of converting a cyano group into an amide group.

9. The variant microorganism as claimed in claim 8, which is a variant strain of a microorganism belonging to the genus *Rhodococcus*.

10. The variant microorganism as claimed in claim 9, which is a variant strain of *Rhodococcus* sp. ATCC39484.

11. A *Rhodococcus* sp. SD826 (FERM BP-7305) strain.

12. A process for producing carboxylic acid, comprising converting a cyano group of a nitrile compound into a carboxyl group using a transformant transformed with a plasmid containing a nitrilase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence encoding the amino acid sequence shown by SEQ ID NO 2 of the sequence list.

13. A process for producing carboxylic acid, comprising converting a cyano group of a nitrile compound into a carboxyl group using a transformant transformed with a plasmid containing a nitrilase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence shown by SEQ ID NO 1 in the sequence list.

14. A process for producing cyano carboxylic acid, comprising converting at least one nitrile group of a polynitrile compound into a carboxyl group using the transformant as claimed in claim 12 or 13.

15. The process for producing cyano carboxylic acid as claimed in claim 14, wherein the polynitrile compound is an aromatic polynitrile compound.

16. The process for producing cyano carboxylic acid as claimed in claim 15,

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wherein the aromatic polynitrile compound is selected from the group consisting of o-phthalonitrile, isophthalonitrile, and terephthalonitrile, and the cyano carboxylic acid is corresponding o- cyanobenzoic acid, m- cyanobenzoic acid, or p-cyanobenzoic acid.

17. A transformant transformed with a plasmid containing a nitrilase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence encoding the amino acid sequence shown by SEQ ID NO 2 of the sequence list, for use in the process as claimed in claim 12 or 13.

18. A transformant transformed with a plasmid containing a nitrilase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence shown by SEQ ID NO 1 of the sequence list, for use in the process as claimed in claim 12 or 13.

19. A plasmid containing a nitrilase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence encoding the amino acid sequence shown by SEQ ID NO 2 of the sequence list, for use in the preparation of the transformant as claimed in claim 17.

20. A plasmid containing a nitrilase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence shown by SEQ ID NO 1 of the sequence list, for use in the preparation of the transformant as claimed in claim 18.

21. A nitrilase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence encoding the amino acid sequence shown by SEQ ID NO 2 of the sequence list.

22. A nitrilase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence shown by SEQ ID NO 1 of the sequence list.

23. The nitrilase gene as claimed in claim 22, wherein the *Rhodococcus* bacterium is *Rhodococcus* sp. ATCC39484 strain.

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24. A process for producing nitrilase, comprising culturing a transformant described in claim 17 or 18 and collecting nitrilase from the culture.
25. Nitrilase prepared by the process as claimed in claim 24.
26. A process for producing amide compound, comprising converting a cyano group of a nitrile compound into an amide group using a transformant transformed with a plasmid containing a nitrile hydratase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence encoding the amino acid sequences shown by SEQ ID NOs 4 and 5 of the sequence list.
27. A process for producing amide compound, comprising converting a cyano group of a nitrile compound into an amide group using a transformant transformed with a plasmid containing a nitrile hydratase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence shown by SEQ ID NO 3 in the sequence list.
28. A process for producing carboxylic acid, comprising converting an amide group of an amide compound into a carboxyl group using a transformant transformed with a plasmid containing an amidase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence encoding the amino acid sequence shown by SEQ ID NO 7 of the sequence list.
29. A process for producing carboxylic acid, comprising converting an amide group of an amide compound into a carboxyl group using a transformant transformed with a plasmid containing an amidase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence shown by SEQ ID NO 6 of the sequence list.
30. A process for producing carboxylic acid, comprising converting a cyano group of a nitrile compound into a carboxyl group using a transformant transformed with a plasmid containing both a nitrile hydratase gene derived from *Rhodococcus* bacterium

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consisting of a DNA sequence encoding the amino acid sequences shown by SEQ ID NOs 4 and/or 5 of the sequence list and an amidase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence encoding the amino acid sequence shown by SEQ ID NO 7 of the sequence list.

31. A process for producing carboxylic acid, comprising converting a cyano group of a nitrile compound into a carboxyl group using a transformant transformed with a plasmid containing both a nitrile hydratase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence shown by SEQ ID NO 3 of the sequence list and an amidase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence shown by SEQ ID NO 6 of the sequence list.

32. The process for producing amide compound as claimed in claim 26 or 27, wherein the nitrile compound is selected from the group consisting of orthophthalonitrile, isophthalonitrile, and terephthalonitrile and the amide compound is corresponding o-cyanobenzamide, m-cyanobenzamide, or p-cyanobenzamide.

33. The process for producing carboxylic acid as claimed in claim 28 or 29, wherein the amide compound is selected from the group consisting of o-cyanobenzamide, m-cyanobenzamide, and p-cyanobenzamide and the carboxylic acid is corresponding o-cyanobenzoic acid, m-cyanobenzoic acid, or p-cyanobenzoic acid.

34. The process for producing carboxyl acid as claimed in claim 30 or 31, wherein the nitrile compound is selected from the group consisting of o-phthalonitrile, isophthalonitrile, and terephthalonitrile and the carboxylic acid is corresponding o-cyanobenzoic acid, m-cyanobenzoic acid, or p-cyanobenzoic acid.

35. A transformant transformed with a plasmid containing a nitrile hydratase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence encoding the

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amino acid sequences shown by SEQ ID NOs 4 and 5 of the sequence list, for use in the process as claimed in claim 26.

36. A transformant transformed with a plasmid containing a nitrile hydratase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence shown by SEQ ID NO 3 of the sequence list, for use in the process as claimed in claim 27.

37. A transformant transformed with a plasmid containing an amidase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence encoding the amino acid sequences shown by SEQ ID NO 7 of the sequence list, for use in the process as claimed in claim 28.

38. A transformant transformed with a plasmid containing an amidase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence shown by SEQ ID NO 6 of the sequence list, for use in the process as claimed in claim 29.

39. A transformant transformed with a plasmid containing both a nitrile hydratase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence encoding the amino acid sequences shown by SEQ ID NOs 4 and/or 5 of the sequence list and an amidase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence encoding the amino acid sequence shown by SEQ ID NO 7 of the sequence list, for use in the process as claimed in claim 30.

40. A transformant transformed with a plasmid containing both a nitrile hydratase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence shown by SEQ ID NO 3 of the sequence list and an amidase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence shown by SEQ ID NO 6 of the sequence list, for use in the process as claimed in claim 31.

41. A plasmid containing a nitrile hydratase gene derived from *Rhodococcus*

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bacterium consisting of a DNA sequence encoding the amino acid sequences shown by SEQ ID NOs 4 and 5 of the sequence list, for use in the preparation of the transformant as claimed in claim 35.

42. A plasmid containing a nitrile hydratase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence shown by SEQ ID NO 3 of the sequence list, for use in the preparation of the transformant as claimed in claim 36.

43. A plasmid containing an amidase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence encoding the amino acid sequence shown by SEQ ID NO 7 of the sequence list, for use in the preparation of the transformant as claimed in claim 37.

44. A plasmid containing an amidase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence shown by SEQ ID NO 6 of the sequence list, for use in the preparation of the transformant as claimed in claim 38.

45. A plasmid containing both a nitrile hydratase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence encoding the amino acid sequences shown by SEQ ID NOs 4 and/or 5 of the sequence list and an amidase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence encoding the amino acid sequence shown by SEQ ID NO 7 of the sequence list, for use in the preparation of the transformant as claimed in claim 39.

46. A plasmid containing both a nitrile hydratase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence shown by SEQ ID NO 3 of the sequence list and an amidase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence shown by SEQ ID NO 6 of the sequence list, for use in the preparation of the transformant as claimed in claim 40.

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47. A nitrile hydratase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence encoding the amino acid sequence shown by SEQ ID NOs 4 and/or 5 of the sequence list.
48. A nitrile hydratase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence shown by SEQ ID NO 3 of the sequence list.
49. The nitrile hydratase gene as claimed in claim 47, wherein the *Rhodococcus* bacterium is *Rhodococcus* sp. ATCC39484 strain.
50. An amidase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence encoding the amino acid sequence shown by SEQ ID NO 7 of the sequence list.
51. An amidase gene derived from *Rhodococcus* bacterium consisting of a DNA sequence shown by SEQ ID NO 6 of the sequence list.
52. The amidase gene as claimed in claim 51, wherein the *Rhodococcus* bacterium is *Rhodococcus* sp. ATCC39484 strain.
53. A process for producing nitrile hydratase, comprising culturing a transformant as claimed in claim 35 or 36 in a culture medium and collecting nitrile hydratase from the culture.
54. A process for producing amidase, comprising culturing a transformant as claimed in claim 37 or 38 in a culture medium and collecting amidase from the culture.
55. A process for producing nitrile hydratase and/or amidase, comprising culturing a transformant as claimed in claim 39 or 40 in a culture medium and collecting nitrile hydratase and/or amidase from the culture.
56. Nitrile hydratase prepared by the process as claimed in claim 53.
57. Amidase prepared by the process as claimed in claim 54.

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58. Nitrile hydratase and/or amidase prepared by the process as claimed in claim

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